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Some Forces that Shape the Nervous System

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ABSTRACT

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Newt embryos are excellent model systems in which to study morphogenesis of the nervous system because there is no growth until larval stages. The forces that shape the neural plate and tube are thus more readily discerned. Jacobson and Gordon, by experiment and computer simulation, have shown that two forces are necessary and sufficient to shape the neural plate into a keyhole shape. These forces are (1) differential and programmed constriction of the apical surfaces of the plate cells and (2) relocation to and along the midline of the neural plate cells that overlie the notochord. The second force is responsible for neural plate elongation and most of the conversion to a keyhole shape, but a normal shape is achieved only when both sets of forces act.

This study examines the role of these two forces in rolling the neural plate into a tube. Both forces continue to function during plate closure. The apical surfaces of the plate cells constrict greatly and the cells become wedge shaped. The change to wedge-shaped cells is believed by many to drive neural plate closure.

The second force – cell relocation over the notochord – occurs at its greatest rate just when the plate rolls into a tube. The nervous system then elongates ten times more rapidly and elongation is achieved by conversion of cross-sectional area to length. An elastic sheet model is presented to show that such elongation can lead to buckling of the sheet out of the plane, the formation of folds, and rolling of the folds into a tube.

Pulsatile narrowing of the neural plate is described and it is suggested that sporadic relaxation of elastic tensions may be responsible. The epidermis is always under tension (not pushing) because the narrowing plate is pulling on it. When slit, the epidermis gapes, thus epidermis resists (not aids) neurulation.

The newt brain expands shortly after tube formation, presumably because cerebrospinal fluid accumulates. Since the brain is not yet growing, expansion would have to be at the expense of wall thickness, and this is confirmed by measurements.

This study examines the shaping processes that occur early in development of the central nervous system including the shaping of the neural plate, the rolling of the plate into a neural tube, and early expansion of the neural tube.

The first indication of nervous system formation in the newt embryo is a change in the behavior of the cells that compose the prospective neural ectoderm. During gastrulation these cells, along with the rest of the ectoderm, move toward the blastopore. As soon as the roof of the archenteron is formed, the dorsal ectoderm ceases these movements of epiboly and most neural ectoderm cells begin to move toward the midline and cephalad. This reversal of direction of cell movement is the visible beginning of neurulation.

The newt neural ectoderm is a sheet of cells one cell thick. The shape of this sheet changes from a hemispheric shell at the end of gastrulation (stage 12) to a flattened keyhole shape at a midneurula stage (stage 15) and then to a neural tube at the end of neurulation (stage 20); stages are those of Twitty & Bodenstein, 1962). When neural tube closure is complete, the brain cavities enlarge and the brain expands. All of this occurs while the neural ectoderm is one cell thick, and in the newt these shaping processes do not involve growth.

Shaping without growth

Since newt embryos have no extracellular yolk or extrinsic food source the embryo as a whole does not grow until it hatches and feeds at stage 40. However, parts of the embryo may grow at the expense of other parts once the circulation is established at early larval stage 34. I have measured the volume of the central nervous system of the California newt, *Taricha torosa*, at the earliest neurula stage 13, at intermediate stages and at early larval stage 33 just before the heart starts to beat. The nervous system does not increase in volume during this period (Fig. 1).

Other mechanisms than growth must be responsible for shaping the nervous system to the early larval stage. Because growth does not obscure the

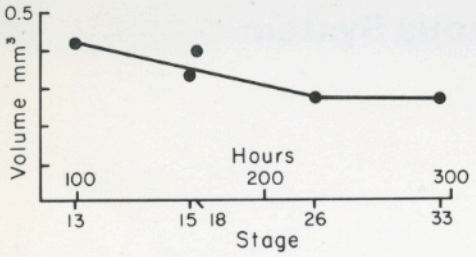


Fig. 1. The volume of the entire nervous system was measured at different stages. Images of the nervous system from serial sections were projected and drawn on paper then cut and weighed to calculate the volumes. There is no growth in the nervous system during these stages.

morphogenetic events that form the complicated larval nervous system, the newt embryo is a valuable model system in which to study shaping of the nervous system.

The two forces that shape the early neural plate

A. G. Jacobson & Gordon (1976 *a, b*) have made an experimental, observational, and mathematical analysis and a computer simulation of the change in shape of the newt neural plate from when it first appears as a hemispheric shell at stage 13 to when it has acquired a flattened keyhole shape at stage 15 just before it begins to form into a tube. The joint operation of two physical forces is necessary and sufficient to accomplish this early shape transformation. The first force is shrinkage of the neural plate surface brought about by contraction of rings of microfilaments near the apical ends of the plate cells. The second force is rearrangement of those neural plate cells that overlie the notochord. These cells change neighbors (shear) and relocate toward and along the midline.

As the apical ends of the plate cells shrink, there is an inversely proportional increase in cell height. These changes are regionally programmed by stage 13, long before they are expressed (A. G. Jacobson & Gordon 1976*a, b*). The contraction of the purse string of microfilaments is transmitted to surrounding cells since the microfilaments attach to the desmosomes that bind cell to cell near their apical ends (Burnside 1971, 1973). Concomitant cell elongation requires microtubule activities. Burnside (1973) has made a detailed study of microfilaments and microtubules in the newt neural plate and suggests that the microtubules probably function by directing cytoplasmic flow basally.

To measure changes of shape in cells, the movements of the cells must first be mapped. Only then can the shapes of the same cells be compared at the beginning and end of a time period.

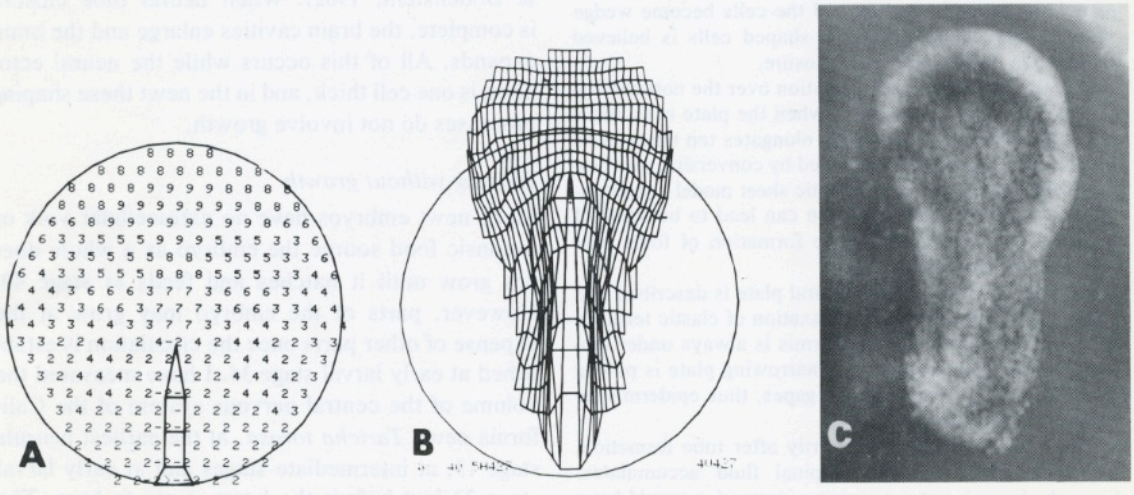


Fig. 2. (*A*) On the circular outline of a stage 13 embryo (D=2.5 mm), numbers are placed that index how much cells in each region will contract their apical surfaces (and elongate) by stage 15. Cells indicated by higher numbers contract more than those with lower numbers. The rocket-shaped region (*bottom of figure*) outlines the supranotochordal cells of the neural plate. This region narrows and elongates in the midline. A computer simulation (Jacobson & Gordon, 1976 *a*) that played out the regional contractions and the relocation of the supranotochordal region produced a change of shape (*B*) that is comparable to the keyhole shape attained by stage 15 in a neural plate isolated with underlying notochord (*C*).

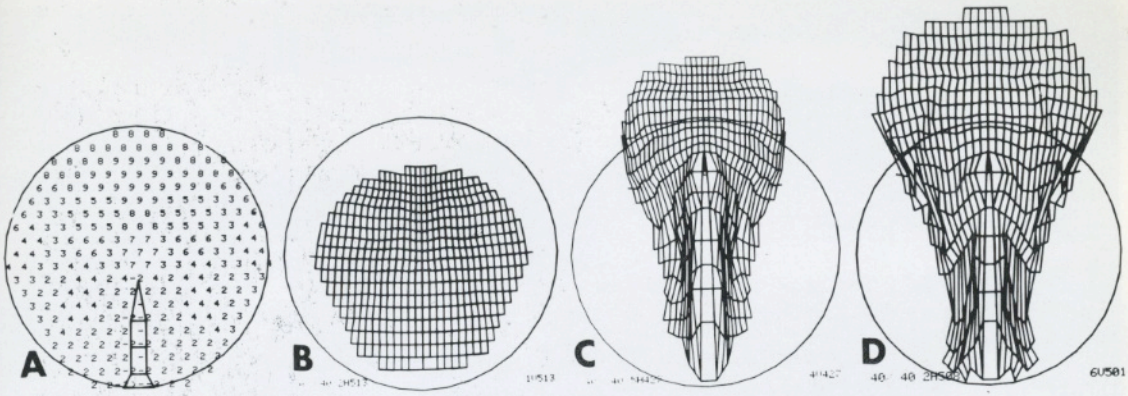


Fig. 3. These photographs of a computer graphics terminal (modified from A. G. Jacobson & Gordon 1976*a*) compare the shapes attained by computer simulation, starting with the stage 13 shape (*A*), with just shrinkage (*B*), with both shrinkage and supranotochordal relocation (the normal case) (*C*), and with just supranotochordal relocation (*D*).

Others (Baker & Schroeder, 1967; Schroeder, 1970) have discussed "cell shape changes" in representative cross sections of anuran embryos (*Hyla regilla, Xenopus laevis*) whose cell movements in the neural plate had not been mapped. Cross sections from similar levels of the embryo at different times will not have the same neural cells in the section, so these authors described shape changes in different cells.

Cells move considerable distances in a complex spatial pattern during formation of the neural plate. C.-O. Jacobson (1962) illustrated such movements between stages 13 and 16 in the axolotl embryo. Burnside & A. G. Jacobson (1968) found neural plate cells move at speeds of up to 95 μm per hour and are displaced as much as one-third the diameter of the embryo between stages 13 and 15. Movements of cells at the intersections of a superimposed coordinate grid were mapped. The pathways of movements of the cells were very consistent from one embryo to another (Burnside & A. G. Jacobson, 1968).

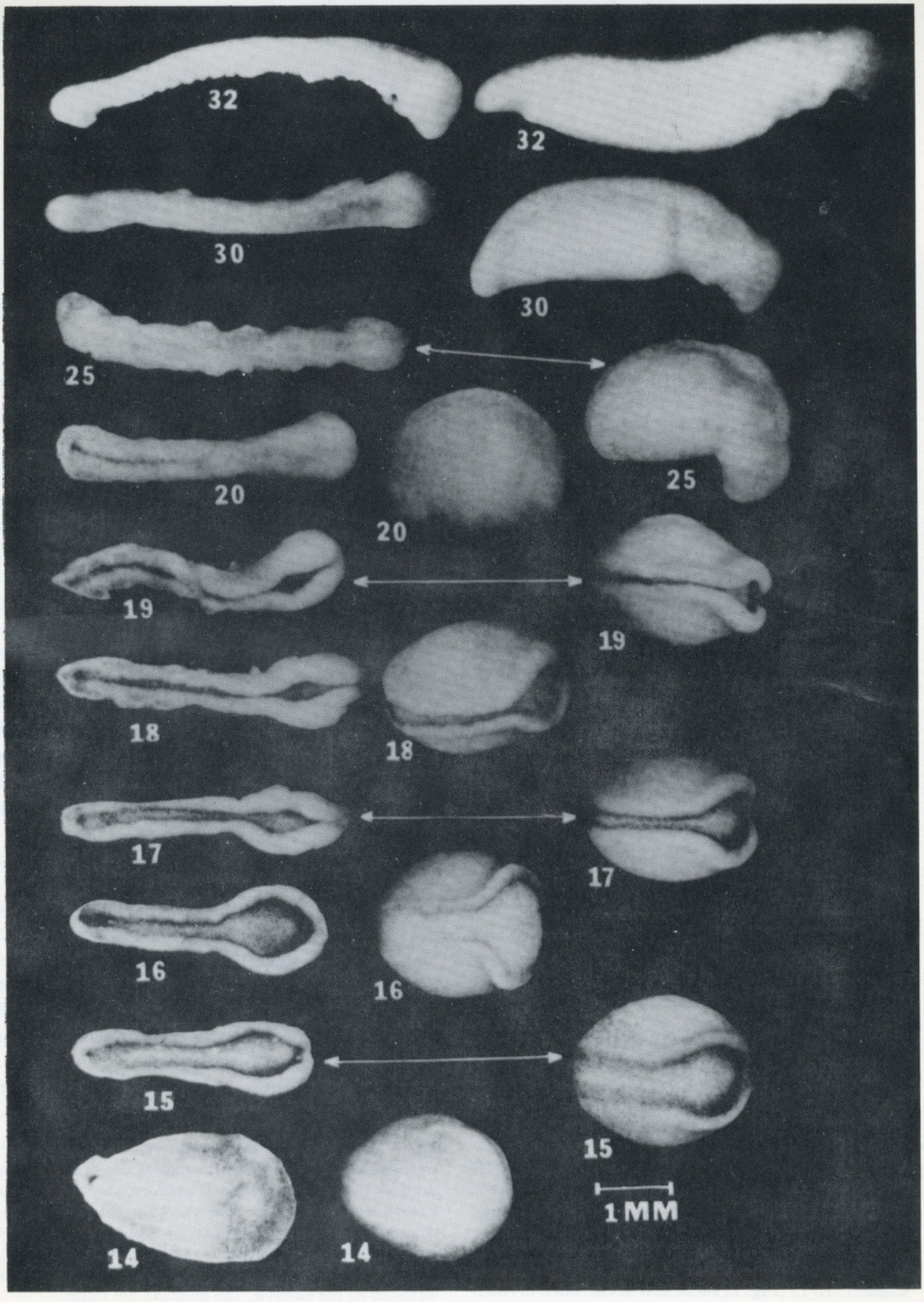
Using this map of cell pathways, detailed cell height measurements have been made at stage 13 and again in the same cell groups, as displaced, at stage 15 (Burnside & Jacobson 1968; Burnside, 1973; Jacobson & Gordon, 1976 *a*). From this data we constructed an empirical map of future changes of cell shape on the stage 13 neural plate (Jacobson & Gordon, 1976 *a, b*). The program of future changes of cell shape was part of the input for a computer simulation of the shaping of the neural plate from stages 13 to 15 (Fig. 2; Jacobson & Gordon, 1976 *a*).

The second force, the relocation of supranotochordal cells, was also empirically defined from observations from time-lapse movies of newt neurulation. We (A. G. Jacobson & Gordon, 1976*a*) found that these cell movements require the presence of both the notochordal and supranotochordal cells, at least during the time when these superimposed sheets of cells are rearranging from a crescent shape near the blastopore at stage 13 to a rod shaped region in the midline at stage 15. It is at stage 15 that the notochord commences to form a sheath, and subsequent notochordal elongation is by a different method involving the formation and enlargement of vesicles and the constraint of the sheath (Mookerjee et al., 1953).

We assessed the relative roles of the two driving forces on early plate shaping (Jacobson & Gordon, 1976 *a*). The rearrangement of supranotochordal cells to the midline can be turned off in the embryo by isolating the stage 13 neural plate without notochord. The plate then shrinks, but does not elongate or convert to a keyhole shape. Similar results were obtained with the computer simulation by setting notochordal changes to zero.

There is no apparent way to stop the shrinkage

Fig. 4. Taricha torosa embryos at various stages (*right*) were photographed, then their neural plates or tubes were excised, laid flat on agar, and photographed within one minute of excision (*left*). The lengths of the nervous systems were then measured from photographs with a ruler or map measurer. This method avoids the optical foreshortening encountered when the nervous systems are wrapped on the embryo. The *numbers* are stages.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program of a living neural plate without affecting other processes. However, the experiment can be done with the computer simulation by setting zero change in cell height and apical shrinkage. The effects of relocation of the supranotochordal cells is then played out alone, producing a keyhole shaped plate that is abnormally long (Fig. 3).

The closing of the plate into a neural tube

The two forces that shape the early neural plate continue to operate as the plate closes into a tube. How might one or both of these forces drive the closing of the tube?

The contraction of the apical surfaces of the neural plate cells continues after stage 15 (in *Taricha torosa;* Burnside, 1973) but the elongation of the cells does not keep pace so some of the cells become flask shaped.

Lewis (1947) gave evidence, with a physical model, that tension on one surface of adherent segments that resist distortion will result in a concave depression on the side of the greater tension. The appearance of flask shaped cells suggests, then, that apical surface contraction can contribute to tube formation. However, the flask shape might also be assumed to be a passive response to other forces that roll up the tube.

I noted above (Fig. 3 *B*) that shrinkage of the neural plate surface continues in neural plates isolated without underlying notochord, but such plates

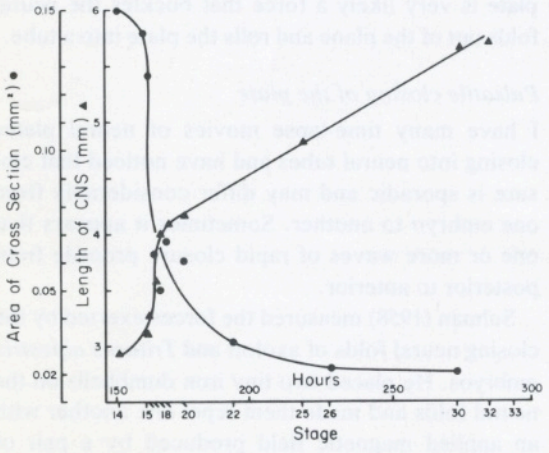

Fig. 5. The change in length of the neural plate or tube (*triangles*) is compared to the cross sectional area of plates or tubes (*points*) measured from sections taken midway along the antero-posterior axis. Length measurements were on preparations as illustrated in Fig. 4.

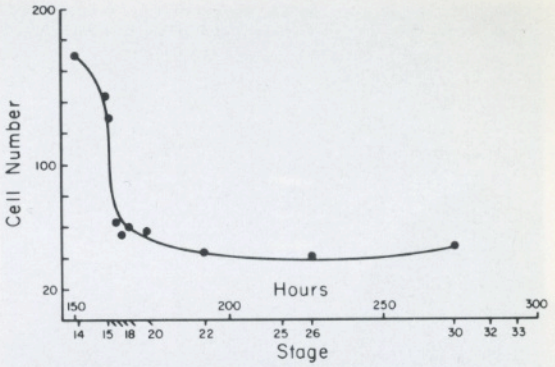

Fig. 6. On the same cross sections of neural plates and tubes used for area measurements in Fig. 5, total cell number was measured counting nuclei.

do not form a tube even when cultured past the time when controls have formed a tube. This suggests that contraction of apical cell surfaces is not the important or only force that causes tube formation.

The second force that shapes the early neural plate – the relocation of supranotochordal cells to and along the midline – is the force responsible for elongation of the early neural plate (Jacobson & Gordon, 1976 *a*). This cell behavior continues to operate with greater intensity during neural plate closure and may be a main force that brings about neural tube formation.

A. G. Jacobson & Gordon (1976 *a*) surgically extirpated entire nervous systems of newt embryos of different stages. The excised neural plates or tubes were laid flat on a bed of neutral agar, photographed (Fig. 4), and their lengths measured. This method eliminated the optical foreshortening encountered when the nervous systems are wrapped about spherical embryos.

The rate of elongation of the nervous systems abruptly increases ten-fold through the period that the neural plate rolls into a neural tube (from stages 15 to 20, Fig. 5; Jacobson & Gordon, 1976 *a*).

The neural plate elongates as a result of the relocation to positions along the midline of cells that overlie the notochord. Relocation of these supranotochordal cells also causes displacement along the antero-posterior axis of the adjacent neural plate cells lying off the midline; the effect decreasing laterally (Jacobson & Gordon, 1976 *a*). The neural plate is essentially adding length at the expense of cross-sectional area with most cell displacement occurring at and near the midline.

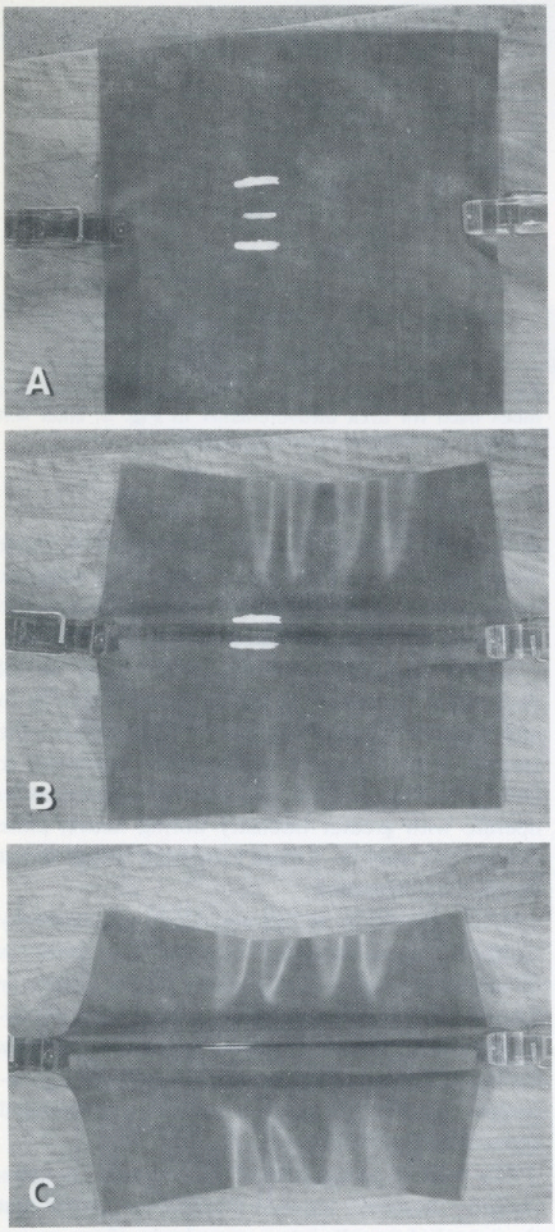

Fig. 7. A piece of rubber dental dam 15 mm square (*A*) was stretched along a line. A fold is raised on each side of the line (*B*). Additional stretching causes the folds to roll toward one another until they meet and form a tube (*C*). The *white lines* were painted on the rubber sheet to provide landmarks.

Measurements of areas of cross-sections confirm this. The area of a cross section at a midpoint along the antero-posterior axis varies through time inversely with the length of the nervous system (Fig. 5). Cell number decreases in cross sections in the same way area does (Fig. 6). Cell number was determined by counting nuclei in each cross section.

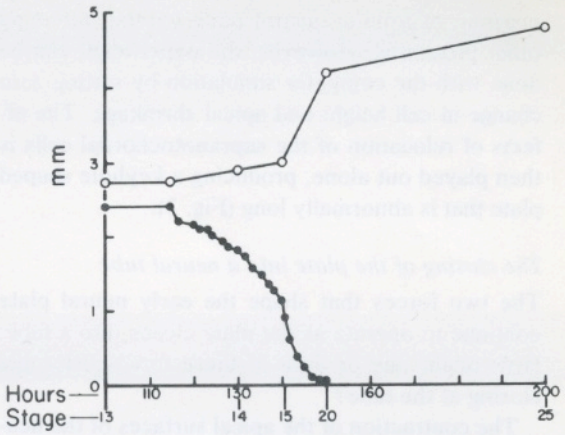

Fig. 8. Neural plate (and tube) length increase through time (*circles*) is compared to the decrease in width of the neural plate at the spinal cord-brain boundary (*points*).

How can this vigorous elongation of the neural plate raise neural folds and close the plate into a tube?

If an elastic sheet is distorted by being stretched along a line, the sheet buckles out of the plane. Ridges rise on each side of a valley that runs along the line of stretch (Fig. 7*B*). If stretching is continued, the ridges roll toward the midline of the valley and may meet to form a tube (Fig. 7 *C*).

The neural plate and attached epidermis are an elastic sheet, so elongation of the midline of the plate is very likely a force that buckles the neural folds out of the plane and rolls the plate into a tube.

Pulsatile closing of the plate

I have many time-lapse movies of neural plates closing into neural tubes and have noticed that closure is sporadic and may differ considerably from one embryo to another. Sometimes it appears that one or more waves of rapid closure procede from posterior to anterior.

Selman (1958) measured the forces exerted by the closing neural folds of axolotl and *Triturus alpestris* embryos. He placed two tiny iron dumbbells on the neural folds and made them repel one another with an applied magnetic field produced by a pair of electromagnetic coils. By varying the current, the force of repulsion could be balanced against the force of neurulation. Selman noted that closure took place in steps, each about 13 μm.

I have measured the width of the neural plate at the prospective brain-spinal cord boundary on projected time-lapse movies of *Taricha torosa* neurulation. There is a correlation of neural plate width decrease and neural plate length increase through time (Fig. 8). More details of this correlation become apparent when the rates of change are compared (Fig. 9). To collect the data for figures 8 and 9, the width of the neural plate was measured at 100 minute intervals. Even at these infrequent intervals periodic fluctuations of rate of width change are seen after stage 14.

A more detailed study of plate width change was made at 6 minute intervals between stage 16 and 18 (Fig. 10). The periodic fluctuations in rate of width decrease occur at 18 to 24 minute intervals (at 17°C) through this period.

In contrast to the sporadic way the width changes, the length of the neural plate appears to increase smoothly except for the abrupt transition to a tenfold faster rate of elongation at stage 15 when tube formation starts, and an abrupt return to the old rate of elongation at stage 20 when tube formation is complete.

One can speculate that neural plate width changes sporadically as a consequence of building up, then sudden release of elastic forces (e.g. in the epidermis) that resist neurulation.

Does the epidermis push the neural folds?

That the epidermis is resisting neurulation, not assisting it, can be demonstrated by gaping experiments such as those done by Karfunkel (1974), and A. G. Jacobson & Gordon (1976 *a*). A small slit made in the epidermis always results in immediate

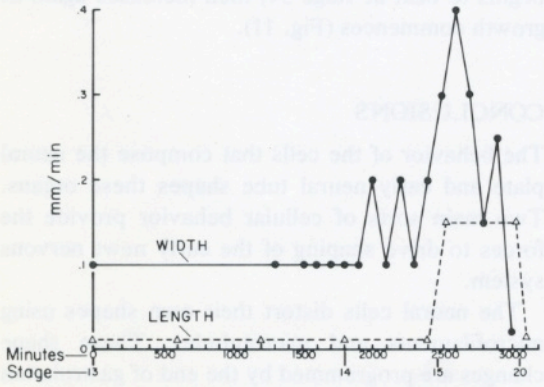

Fig. 9. The *rate* at which the width of the neural plate decreases through time (*solid line*) is compared to the *rate* at which the nervous system elongates (*dashed line*).

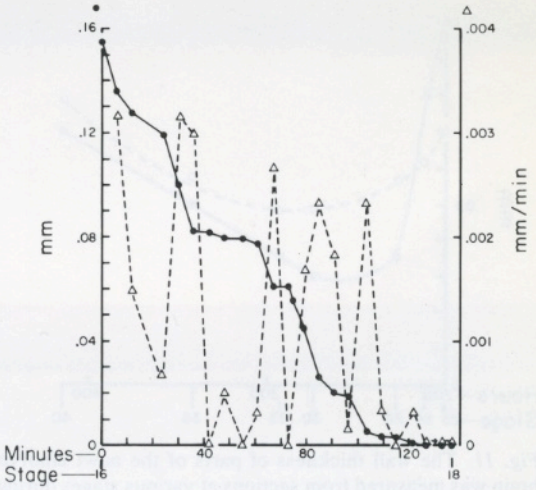

Fig. 10. The width of the brain-spinal cord boundary of the neural plate was measured every six minutes from stages 16 to 18 (*solid line*). The rate of change of width (*dashed line*) peaks every 18 to 24 min at 17°C.

gaping so the instantaneous forces in the epidermis are those of tension, not compression. Once wounded, the epidermis has a free edge and wound closure begins to pull the edges together to close the gaping wound. Closure of the wound is usually complete within an hour.

Selman (1958) found the neurulation forces measured with his small magnets were less if the epidermis lateral to the fold was slit. I suspect the reason for his results is that neural plate is under elastic tension due to the resistance of the epidermis. When freed from the epidermis, these elastic forces are released and the plate instantly narrows. This narrowing can be seen in the excised plates of stages 15, 16, 17 and 18 in Fig. 5 (the plates were photographed within one minute of excision). Selman probably made his measurements just after the plate was relaxed.

A. G. Jacobson & Gordon (1976 *a*) made slits in the intact neural plate and in the plate just after excision. We found that the former gaped while the latter did not. The tension had relaxed.

For the above reasons, I believe the papers of those authors that invoke pushing by the epidermis as contributory to normal neurulation (Giersberg, 1924; Selman, 1955; Schroeder, 1970; C.-O. Jacobson & A. Jacobson, 1973) are open to reinterpretation.

Gordon and I (A. G. Jacobson & Gordon, 1976*a*) found that neural plates isolated at stage 13 with no

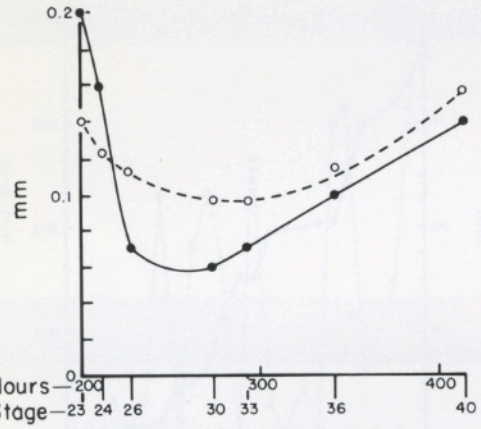

Fig. 11. The wall thickness of parts of the newt embryo brain was measured from sections at various stages during brain expansion. Diencephalon wall (*solid line*) was measured above the attachment of the optic stalk. Diencephalon enlarges more than mesencephalon (*dashed line*) during this period.

other tissue than the attached underlying notochord will form neural tubes. Neither epidermis nor any mesoderm other than notochord is necessary for neurulation.

Lines of shear and cell domains

A. G. Jacobson & Gordon (1976 *a*) found that the boundary between the neural plate and epidermis must be a line of shear (where cells change neighbors), as is the midline region of the supranotochordal cells. We suggested that these lines of shear may function in the isolation of cell domains. The shear at the midline may define, or help define, the bilateral symmetry of the neural plate and the shear at the border of the plate may help define and isolate the cellular domains of neural plate and epidermis. We suggested that junctional communication (as described by Loewenstein (1968) and Furshpan & Potter (1968)) may be disrupted between the groups of cells by the lines of shear.

Burnside & A. G. Jacobson (1968) analyzed high magnification time-lapse movies that showed apical cell boundaries in the lateral brain plate region of open neural plate stages. This region was chosen for these difficult high magnification studies because the area is uppermost as the embryo orients itself in the water. These cells did not change neighbors. One sentence in the summary of our paper left the impression that no neural plate cells change neighbors and that is incorrect.

Other lines of shear emerge during later morphogenesis of the central nervous system. We have preliminary evidence that a line of shear appears at the boundary between prospective sensory retina and prospective pigmented retinal epithelium in the forming optic cup. The gradual delimitation and subdivision of embryonic fields in the brain plate may be facilitated by lines of shear.

Expansion of the brain

Desmond & A. G. Jacobson (1977) have shown that early brain enlargement in the chick embryo requires cerebrospinal fluid pressure. When the brain ventricles of the chick become a closed system and the brain enlarges, the heart has been beating for some time. The early chick embryo is a growing system.

Intubation of young chick embryo brains relieves cerebrospinal fluid pressure and the brains then expand very little. The nervous tissue continues to divide and to grow some and folds into the ventricles (Desmond & Jacobson, 1977).

The newt neural tube is a closed tube by stage 23 and brain expansion starts immediately. If cerebrospinal fluid pressure is a factor, then the fluid must be accumulating by secretion in early stages since there is, as yet, no circulation or blood pressure. Since the newt nervous system is not growing at these stages, this early expansion of the brain must be at the expense of wall thickness.

I have measured the thickness of the diencephalon wall (just dorsal to the optic stalk attachment) and of the mesencephalon wall between stages 23 to 40. Brain expansion occurs through the whole period, but wall thickness decreases until the heart begins to beat at stage 34, then increases again as growth commences (Fig. 11).

CONCLUSIONS

The behavior of the cells that compose the neural plate and early neural tube shapes these organs. Two main sorts of cellular behavior provide the forces to drive shaping of the early newt nervous system.

The neural cells distort their own shapes using microfilaments and microtubules. These shape changes are programmed by the end of gastrulation to occur in different amounts in the various regions of the neural plate.

The other cellular behavior that drives plate and

tube shaping is the relocation of cells overlying the notochord to and along the midline. These cellular relocations elongate the plate, especially rapidly during tube formation. It is this force that is mainly responsible for shaping the plate into a keyhole shape and then into a tube, but a normally shaped nervous system results only when both forces act normally.

During neural plate and tube formation, the narrowing plate pulls on the epidermis which offers an elastic resistance to neurulation that may be relieved sporadically.

Once the neural tube is formed, another force – cerebrospinal fluid pressure – is an essential force for normal brain expansion.

In other embryos, such as birds and mammals, growth begins early in the nervous system and shaping due to growth processes overlaps with the operation of the forces described above. In these embryos and in older newt larvae differential mitosis (followed by daughter cell enlargement), selective cell death, and various sorts of cell migration become very important shaping forces.

ACKNOWLEDGEMENT

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